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Expression of the SART-1 Antigens in Uterine Cancers

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We recently reported that the *SART-1* gene, encoding the SART-1_{259} tumor antigen which is recognized by HLA-A26-restricted cytotoxic T lymphocytes (CTLs), is expressed in the cytosol of squamous cell carcinomas and adenocarcinomas. The present study deals with the expression of SART-1_{259} and SART-1_{800} antigens in uterine cancers. The SART-1_{259} antigen was detected in the cytosol fraction of 4 of 8 uterine cancer cell lines, 24 of 74 (32%) uterine cancer tissues, 0 of 7 uterine myomas, and 0 of 5 non-tumorous uterine tissues. The SART-1_{800} antigen was expressed in the nuclear fraction of all the uterine cancer cell lines, 41 of 74 (55%) uterine cancer tissues, 0 of 7 myomas, and 3 of 5 non-tumorous uterine tissues. The SART-1_{259}^{+} uterine cancer cells were recognized by HLA-A24 restricted and SART-1 specific CTLs. Therefore, SART-1_{259} antigen could be an appropriate vaccine candidate for a relatively large number of uterine cancer patients.

Key words: SART-1 antigen — Uterine cancer — Cytotoxic T lymphocyte — Immunotherapy — Cancer vaccine

Many genes encoding tumor-rejection antigens recognized by cytotoxic T lymphocytes (CTLs) have been identified in the cDNA of melanomas.[1–3] Some peptides encoded by these genes are under clinical trial as cancer vaccines and major tumor regression has been seen in melanoma patients.[4–6] Although infiltration of $CD8^+$ T cells in dysplastic uterine tissue has been reported,[7] little is known about the molecular basis of the host defense against cancer cells in uterine cancer patients. A literature search revealed no information on tumor antigens available for specific immunotherapy of uterine cancer. We recently reported a *SART-1* gene encoding tumor antigens recognized by HLA-A26-restricted CTLs.[8,9] The *SART-1* gene encoded both the 125 kD SART-1_{800} antigen expressed in the nucleus of the majority of proliferating cells and the 43 kD SART-1_{259} antigen in the cytosol of the majority of squamous cell carcinomas (SCCs) and some adenocarcinomas, though it does not present itself in other types of cancers or in any normal cells. In this study, we investigated the expression of SART-1 antigens in uterine cancers.

MATERIALS AND METHODS

Samples Uterine cancer tissues (n=74), benign uterine tumor (myoma) tissues (n=7) and non-tumorous uterine tissues (n=5) were obtained by surgical removal either in the Kurume University Hospital or in the Kyoundo Hospital. A section of each sample was minced with scissors and frozen at −80°C until use. A total of 74 uterine cancer tissues consisted of 37 cervical cancers and 37 endometrial cancers. The 37 cervical cancers consisted of 28 SCCs, 7 adenocarcinomas, and 2 adenosquamous cell carcinomas. The 37 endometrial cancers consisted of 36 adenocarcinomas and 1 clear cell carcinoma. Eight cervical cancer cell lines (SKG-1, -2, -3a, -3b, OMC-1, -4, TCS, and HCS), and the KE4 esophageal tumor cell line (HLA-A2402/A2601), from which the *SART-1* gene was cloned as a positive control, were also studied.

Detection of the SART-1 antigens Tissues were sonicated for 60 to 90 s in an Astron ultrasonic processor (Heat Systems, Farmingdale, NY). As previously reported,[9] expression of SART-1_{259} and SART-1_{800} antigen in the samples was analyzed by western blot analysis with polyclonal anti-SART-1_{259} and anti-SART-1_{800} antibody, respectively,

CTL assay The HLA-A24-restricted and SART-1 specific CTL line (1×10^5 cells/well) was newly established from the peripheral blood mononuclear cells (PBMCs) of an esophageal cancer patient (Kikuchi *et al.*, manuscript under submission). Briefly, the patient's PBMCs were stimulated with the autologous tumor cell line (KE4) in a medium (45% RPMI-1640 medium, 45% AIM-V medium [GIBCO BRL, Walkersville, MA], 10% fetal calf serum [EQUITECH BIO, Ingram, TX] with 100 units/ml of IL-2 [Shionogi Pharm. Co., Osaka] and 0.1 m*M* nonessential amino acids solution [GIBCO BRL]). The specificity of this CTL line was confirmed by a 6 h ^{51}Cr-release assay[8] using HLA-A2402 positive and/or negative normal and/or tumor cell lines.

The cervical cancer cell line cells were stained with anti-HLA-A24 monoclonal antibody (One Lamda, Inc.,

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Canoga Park, CA) and the expression of HLA-A24 was measured with a FACScan (Becton Dickinson, San Jose, CA) as reported.[8] The cancer cells (1×10^4 cells/well) were added to each well of a 96-well microtiter plate and were cultured at 37°C for 24 h. The HLA-A24-restricted and SART-1 specific CTL (1×10^5 cells/well) were added to each well, and then cultured for 24 h. The supernatant was collected to measure interferon (IFN)-γ by means of ELISA (Otsuka Pharm. Co., Tokyo) in a triplicate assay.[9]

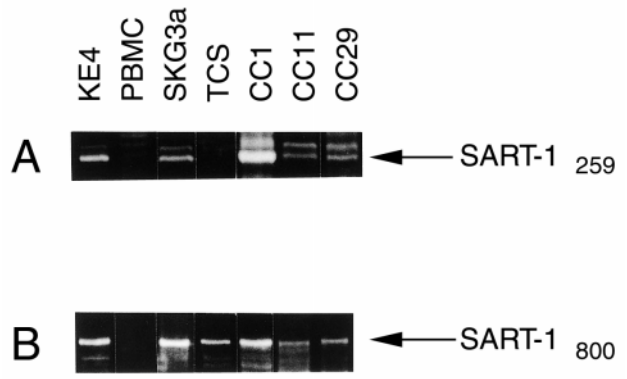

Fig. 1. Expression of the SART-1_{259} and SART-1_{800} antigens. Expression of the SART-1_{259} and SART-1_{800} antigens at the protein level was investigated by western blot analysis using (A) anti-SART-1_{259} and (B) anti-SART-1_{800} polyclonal antibodies as previously described.[9] Cytosol and nuclear fractions were both investigated. Representative results are shown in this figure. PBMC, a negative control; KE4, an esophageal SCC as a positive control; SKG3a, TCS, cervical cancer cell lines. CC1, CC11, and CC29: uterine cancer tissues.

RESULTS

The expression of the SART-1 antigens on malignant and normal uterine cells and tissues was investigated by western blot analysis. Representative results are shown in Fig. 1, and a summary is shown in Table I. The SART-1_{259} antigen was expressed in the cytosol fraction of 4 of 8 (50%) cervical cancer cell lines, 13 of 37 (35%) cervical cancer tissues, and 11 of 37 (30%) endometrial cancer tis-

Table I. Expression of the SART-1_{259} and the SART-1_{800} Proteins

	SART-1_{259} (%)	SART-1_{800} (%)
Cell lines	4/8 (50)	8/8 (100)
Cervical carcinoma	13/37 (35)	20/37 (54)
Endometrial carcinoma	11/37 (30)	21/37 (57)
Myoma	0/7 (0)	0/7 (0)
Non-tumorous tissue	0/5 (0)	3/5 (60)

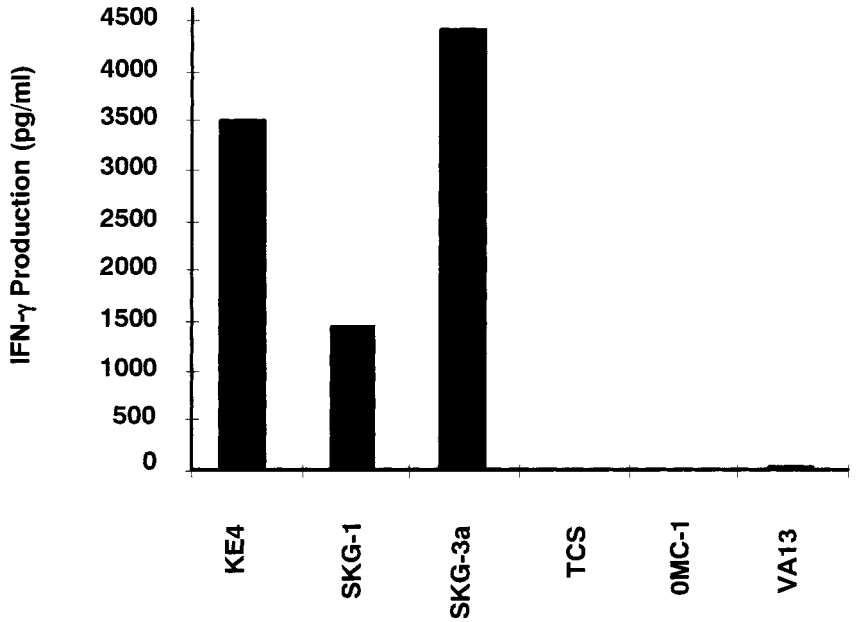

Fig. 2. Recognition of the SART-1_{259} antigen by the CTLs. SKG-1, SKG-3a, and TCS cervical tumor cells (HLA-A24$^+$), and OMC-1 cervical tumor cells and VA13 (HLA-A24$^-$) were tested for the ability to stimulate IFN-γ production by the HLA-A24-restricted and SART-1-specific CTLs that were established from the PBMCs of an esophageal cancer patient at an effector-to-target cell ratio of 5. Values represent the mean of the triplicate assays.

Table II. Expression of the HLA-A24, the SART-1_{259} and the SART-1_{800} Proteins

	HLA-A24	SART-1_{259}	SART-1_{800}
KE4	+	+	+
SKG-1	+	+	+
SKG-3a	+	+	+
TCS	+	−	+
OMC-1	−	−	+
VA13	−	−	+

sues. The SART-1_{259} antigen was not detectable in either benign tumors or in normal tissues. In a histological classification, the antigen was detected in 9 of 28 (32%) SCCs, and 11 of 43 (26%) adenocarcinomas. In all samples tested, SART-1_{259} was undetectable in the nuclear fraction. The SART-1_{800} antigen was expressed in the nuclear fraction of all of 8 cervical cancer cell lines, 20 of 37 (54%) cervical cancers, 21 of 37 (57%) endometrial cancer tissues, 0 of 7 benign uterine tumor tissues, and 3 of 5 non-tumorous uterine tissues. It was not expressed in the cytosol fraction of any of the samples. In a histological classification, the SART-1_{800} antigen was expressed in 17 of 28 (61%) SCCs, and in 23 of 43 (53%) adenocarcinomas. There was no obvious correlation between the expression of the SART-1 antigens and the clinical stage or histological grade of the tumors (data not shown).

To understand whether the SART-1 antigen in uterine cancer cells was recognized by the CTLs, 4 cervical cancer cell lines were tested for the ability to stimulate IFN-γ production by HLA-A24 restricted and SART-1 specific CTLs. The SKG-1, SKG-3a, and KE4 tumors as positive controls stimulated significant amounts of IFN-γ production by the CTLs (Fig. 2, Table II). These three tumor cells were SART-1_{800}^{+}, SART-1_{259}^{+}, and HLA-A24^{+}. In contrast, TCS (SART-1_{800}^{+}, SART-1_{259}^{-}, HLA-A24^{+}), OMC-1, and VA 13 cells (SART-1_{800}^{+}, SART-1_{259}^{-}, and HLA-A24^{-}) failed to stimulate IFN-γ production.

DISCUSSION

SART-1_{800} is a nuclear protein expressed in all proliferating cells, both malignant and normal, including phytohemagglutinin-blasts.[9] Recent work has shown that SART-1_{800} is a cell-cycle dependent protein detectable only at the M-phase (Imai *et al.*, unpublished results). These results suggest that the SART-1_{800} protein is detectable when the sample in question contains a reasonable number of proliferating cells under mitosis. Alternatively, SART-1_{800}-negative tumors might contain too few proliferating cells for detection with western blot analysis under the conditions used. This assumption is supported by the present results that SART-1_{800} was expressed in half of both non-tumorous uterine tissues and uterine cancer tissues. Some normal uterine tissue contains proliferating cells under mitosis. In contrast, SART-1_{800} was undetectable in all of the myomas tested, suggesting a decrease in growth tendency.

The SART-1_{259} antigen was detectable in one-third of uterine cancer tissues sampled. Histologically, it was detected in 10 of 28 (36%) SCCs, and in 11 of 43 (26%) adenocarcinomas. The SART-1_{259} antigen was expressed in the majority of head and neck SCCs, 60% of esophageal SCCs, half of lung SCCs and adenocarcinomas,[9] and 21% of ovarian adenocarcinomas (Shichijo *et al.*, unpublished results). It was not expressed at all in breast cancer,[10] melanomas, or leukemic cells.[9] These results suggest that levels of SART-1_{259} expression vary among the epithelial cancers, largely because of a relatively higher expression of SART-1_{259} in SCCs as compared to that in adenocarcinomas.

The SART-$1_{737\text{-}744}$ peptide that is recognized by HLA-A26-restricted CTLs has the ability to induce CTLs in PBMC. Therefore, it is a vaccine candidate for HLA-A26 and SART-1_{259}^{+} cancer patients.[9] The HLA-A26 allele is found in 22% of Japanese, 17% of Caucasians, and 16% of Africans.[11] Furthermore, we recently found that the SART-1_{259}-derived peptide was recognized by the HLA-A24-restricted CTL line that was newly established from an esophageal cancer patient (Kikuchi *et al.*, unpublished results). This CTL line recognized the SART-1_{259}^{+} uterine cancer cells in an HLA-A24-restricted manner. The HLA-A24 allele is found in 60% of Japanese, 20% of Caucasians, and 12% of Blacks.[11] Therefore, the SART-1_{259} antigen and its peptides could be an appropriate vaccine candidate for a relatively large number of potential uterine cancer patients.

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